



Quantitative ELISA of Polychlorinated Biphenyls in an Oily Soil Matrix

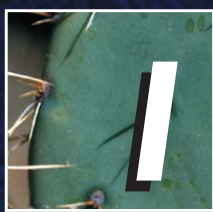
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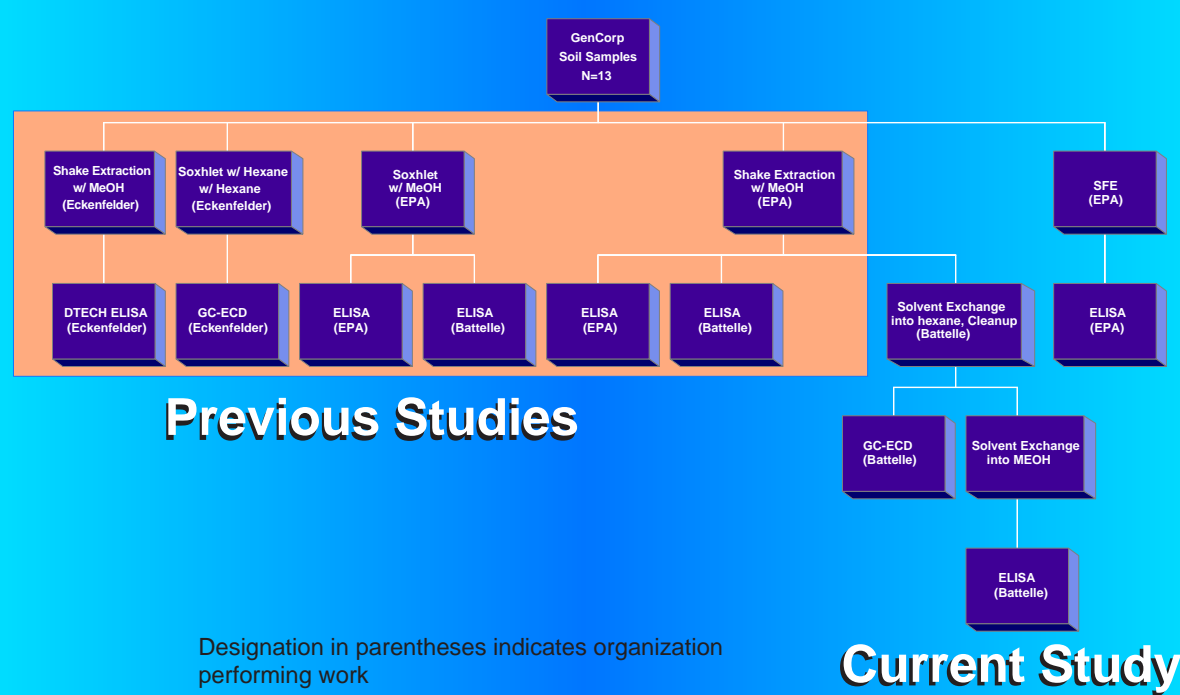


Background

Soil samples were collected at the GenCorp Lawrence Brownfield site, and previously analyzed with a commercial, semi-quantitative homogeneous immunoassay (DTECH) and a quantitative 96-well plate ELISA developed by the EPA^{1,2,3}. Many of these samples were extremely oily, with total petroleum hydrocarbon levels up to 240,000 ppm, as determined by EPA Method 418.1. Both immunochemical approaches, when coupled to abbreviated sample preparation methods, suffered from the inability to handle the oily samples. The commercial method used an extraction consisting of shaking the sample with methanol and stainless steel balls, followed by a filtration. Sample extraction methods for the EPA ELISA consisted of either the shake extraction used for the commercial method, or overnight Soxhlet extraction using methanol. The commercial kit results did not agree with confirmatory results obtained with gas chromatography (GC), whereas the results for the quantitative plate ELISA, for both extraction techniques, agreed reasonably well with GC, except for the more oily samples.

The current work reports our preliminary efforts to examine the effects of different solvents (methanol vs. acetone/hexane) when comparing ELISA data to GC data, and to employ the potential selectivity of supercritical fluid extraction (SFE) to eliminate or reduce spurious ELISA results for oily samples.

2 Study Design



Supercritical Fluid Extraction Methodology

Instrumentation: Suprex AutoPrep 44

Extraction Vessel: 5 mL, silanized glass wool plug placed over frits

Sample Size: 3 g

Sample Preparation: Mixed with anhydrous MgSO₄ until free flowing

Extraction Protocol: 175 ATM equilibration @ 75 °C
250 ATM static for 2 min. @ 85 °C
350 ATM, 1.5 mL/min CO₂ (no modifier) for 20 min.
@ 100 °C

Trapping method: Trap: stainless steel balls @ -30 °C, restrictor @ 50 °C
Desorption: 3 mL methanol, 1 mL/min. @ 40 °C
Flush between samples: 4 mL methanol @ 1 mL/min.

7 ELISA Results For SFE Extracts

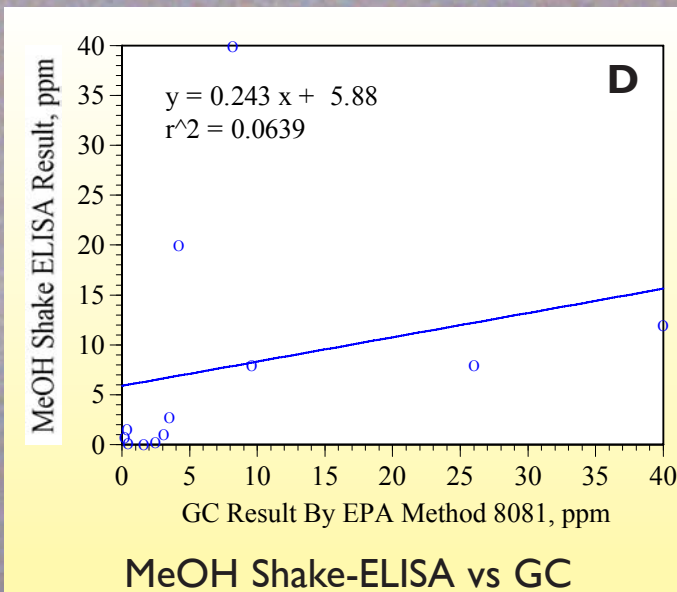
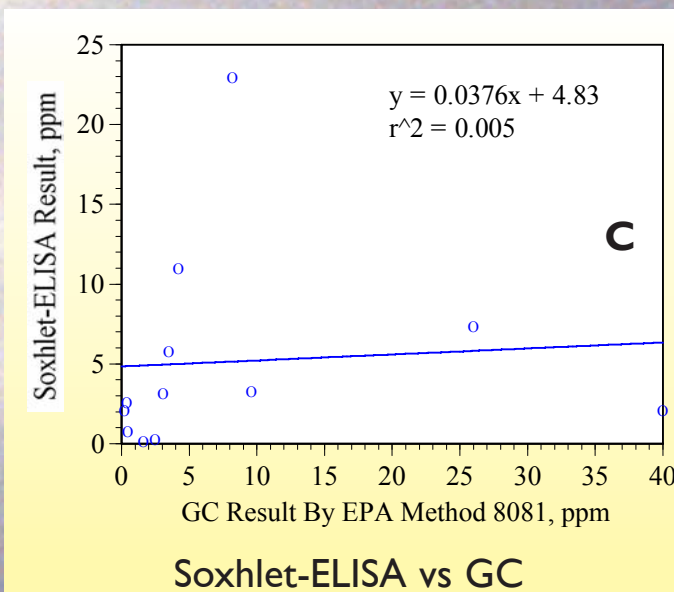
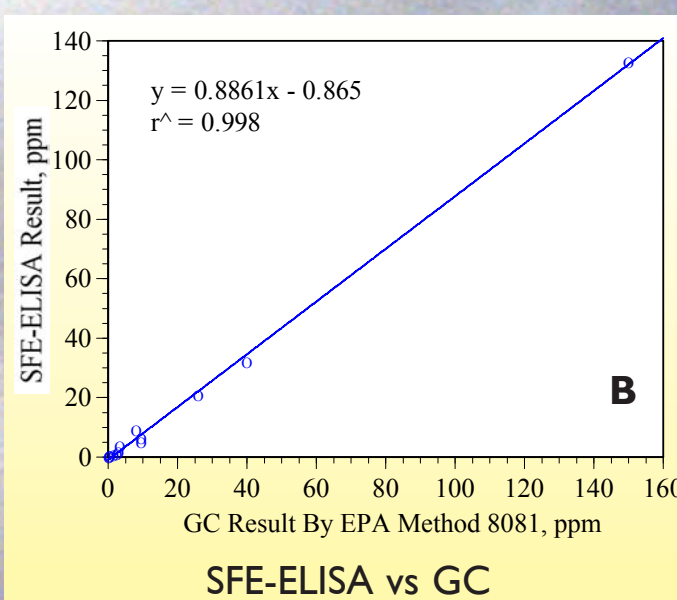
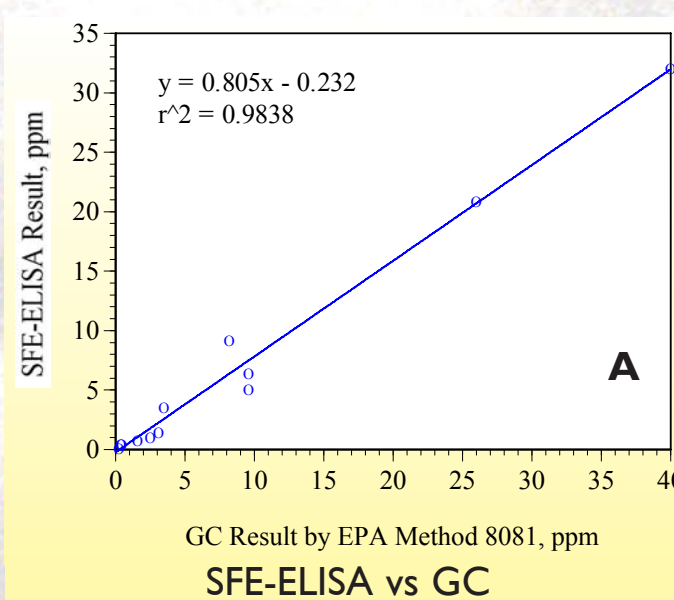
Sample	Method 8081 (GC) Result, ppm	ELISA Result, ppm
CS-001	40	32
CS-004	26	21
CS-008	150	133 (57)*
CS-012	8.2	9.2
CS-015	4.2	5.1
CS-017	9.6	6.5
CS-021	2.5	1.0
CS-022	1.6	0.78
CS-027	0.39	0.49
CS-043	0.22	0.16
CS-045	0.44	0.51
CS-046	3.5	3.6
CS-058	3.1	1.5

* Initial extraction of CS-008 by the method outlined above gave an ELISA result of 57 ppm.

A new sample of CS-008 was re-extracted four times in series using the same extraction conditions outlined above with no flush between extractions. The sum of the ELISA results for the four extracts was 133 ppm. The four extracts contained 59.8, 44.5, 26.4 and 1.83 ppm.

Observations: samples CS-001, 004, 021 exhibited some smaller insoluble oil deposits

Comparison of ELISA Results for Methanolic Shake Extracts, Methanolic Soxhlet Extracts and SFE Extracts to EPA Method 8081 GC Results



3 ELISA Background

- Quantitative, indirect inhibition ELISA formatted into a 96 well plate
- Based on polyclonal rabbit antibodies¹
- Linear working range of 50-1300 ng/mL (7.4-194 ng/mL in actual assay solution)
- Used successfully on a variety of soil matrices¹
- Calibrated with Aroclor 1242 for current study (Aroclors 1242 and 1248 respond nearly identically)

Method Accuracy and Quality Control

Soil standard reference material from Environmental Resource Associates was analyzed by each assay

- Certified value: 18.0 mg/kg Aroclor 1248
- ELISA value: mean = 19.2, sd = 1.87, N = 6
- Demonstrates determinative accuracy of ELISA for "simple" soil matrix



Soil Sample Characteristics

Samples contaminated with Aroclors 1242 and 1248

Samples CS-001 through CS-017, CS-021 visibly oily, verified by analysis for total petroleum hydrocarbons (TPH) by EPA Method 418.1

Sample	TPH, ppm
CS-001	240,000
CS-012	350
CS-017	1000
CS-045	not detected

Samples CS-001, CS-004, CS-008, CS-021 exhibited large globular oil deposits upon cooling after Soxhlet with methanol



Effect of Extraction Method on Determinative Step

Hypothesis: The difference in GC and ELISA results is due to the difference in extraction methods/solvents used for the two determinative methods.

Hypothesis Testing: Extract samples for GC analysis (hexane/acetone), perform cleanup, solvent exchange into MeOH for ELISA.

Actual case: The MeOH extracts used in the ELISA were exchanged into hexane, cleanup was performed followed by GC analysis, and exchanged back into MeOH for ELISA analysis

Results for ELISA and GC Analysis of Solvent Exchanged Extracts

Sample	ELISA Result, ppm	Battelle GC Result, ppm	Eckenfelder 8081 result, ppm
CS-001	63	153	40
CS-004	43	136	26
CS-008	1040	2521	150
CS-012	31	33	8.2
CS-015	36	21	4.2
CS-017	17	23	9.6
CS-021	0.30	1.7	2.5
CS-022	0.10	1.1	1.6
CS-027	2.1	0.50	0.39
CS-043	0.10	0.30	0.22
CS-045	0.20	0.20	0.44
CS-046	3.7	7.8	3.5
CS-058	3.4	6.8	3.1
ERA SRM, 18 ppm	9.6	17	NA

Observations

- Based on the high bias in the ELISA results for the oily samples (CS-001 - CS-017) it appears that the MeOH sample preparation extracted some interfering compound(s) which were not subsequently removed by the clean-up method used in GC analysis.
- The exchange method appears flawed; based on SRM data, there appears to have been a loss of PCBs when going from hexane back to MeOH. This exchange utilized a solvent blow-down followed by replacement with MeOH.
- The GC/GC comparison shows additional error, which can be explained in part by the high bias exhibited by the earlier Battelle GC results.

8 Conclusions

- ELISA results for the supercritical fluid extracts were comparable to Method 8081 gas chromatographic reference data.
- The size of the sample set was admittedly small; the results obtained for the SFE extracts and in our previous work¹ clearly demonstrate the potential utility of coupling SFE with ELISA for samples requiring cleanup.
- This SFE Method was apparently unable to extract high levels (150 ppm) of PCBs in one pass; further investigation of this may be of interest.
- It appears that methanolic extraction may extract compounds which cross-react or interfere with the ELISA such that low results, relative to GC, are produced. It would be interesting to perform a classical hexane extraction, perform the clean up, and then solvent exchange into methanol for ELISA.
- Removal of slight amounts of oil by SFE did not appear to be deleterious, although further work toward optimization of SFE parameters may eliminate this problem.



References

- Johnson, J.C.; Van Emon J.M.; Anal. Chemistry, 1996, 68, 162-169.
- "Reevaluation of Polychlorinated Biphenyl Contaminated GenCorp Lawrence Soil Using Quantitative Immunoassay" presented at the 214th National Meeting of the American Chemical Society Meeting, September 7-11, 1997. Johnson, J.C.; Van Emon, J.M.; Chuang, J.C.; Clarke, A.N.; Wamsley, B.N.
- "Screening of Soils From the GenCorp Lawrence Location for PCBs Using Immunoassay Techniques," Prepared for GenCorp Inc. by Eckenfelder Inc, January 1996.